NEW ZEALAND JOURNAL OF ECOLOGY

RESEARCH

The role of frost tolerance in defining the kauri line floristic boundary

Tobias A W Elliott[1]* , Bruce R Burns[1] , George L W Perry[2] and Cate Macinnis-Ng[1]

[1]School of Biological Sciences, University of Auckland, Private Bag 92019, New Zealand
[2]School of Environment, University of Auckland, Private Bag 92019, New Zealand
*Author for correspondence (Email: tell894@aucklanduni.ac.nz)

Abstract: Floristic boundaries, where the range limits of multiple species coincide, are frequently recognised ecological phenomena for which underlying mechanisms are often unclear. Plant species range limits are often determined by their tolerance to climatic conditions. Therefore, the positions of floristic boundaries are also likely related to climatic conditions. Seedlings are a vulnerable life stage and are often highly susceptible to adverse climatic events such as frosts, and frost susceptibility varies among plant species. Few studies have focussed on the relationship between measured frost tolerances of different plant species and their distributions relative to a floristic boundary. Our study looked at interspecific variation in frost tolerance in a suite of native tree species relative to the kauri line, a floristic boundary recognised at approximately 38°S in Aotearoa New Zealand. We exposed seedlings of four species with range limits near the kauri line and three species with more widespread distributions to one-off simulated frost events and monitored their health for eight weeks after the event. We also excised, froze, and conducted electrolyte leakage tests on leaf samples from each species. The frost tolerances of the kauri line species were similar to each other and were generally lower than those of the widespread species. The outcomes of the electrolyte leakage tests were broadly consistent with the whole seedling frost tests, although the frost tolerance of *Pseudopanax lessonii*, a kauri line species, was under-estimated. Our study highlights that frosts could play a role in the ranges of kauri line species, especially species whose seedlings are common in early successional habitats, such as *Agathis australis*. The convergence of the frost tolerance of *A. australis* with those of other kauri line species is not consistent with the Pleistocene ratchet hypothesis, which suggests a decoupling of plant species current and potential ranges due to rapid climatic changes. Climate change is likely to reduce the incidence of frosts in New Zealand and may facilitate the southward shift of kauri line species, changing the nature of this boundary.

Keywords: *Agathis australis*, electrolyte leakage, leaf traits, seedlings, species ranges

Introduction

Floristic boundaries are biogeographic constructs that describe the coincidence of multiple plant range limits leading to disjunctions in plant distributions (Liu et al. 2023). The factors contributing to the formation and maintenance of these boundaries are complex and often interact (Sexton et al. 2009). Boundary-creating factors likely include historical, environmental, and/or disturbance-related factors (Slik et al. 2011; Ettinger & HilleRisLambers 2013; Esler et al. 2015; Antonelli 2017; Ogden & Perry 2023). Several floristic boundaries have been recognised in Aotearoa New Zealand (Cockayne 1917; Wardle 1963; McGlone 1985). For example, Wardle (1963) identified six boundaries, including the Taupō line at 39°S and a line in the northern South Island at c. 42°S. The kauri line at 38°S is another such boundary and was recognised by Bartlett (1976) and McGlone (1985). This boundary corresponds with the southern natural limit of *Agathis australis* (D. Don) Lindl. Ex Loudon (kauri), a large pioneering conifer (Ogden et al. 1992). Canopy tree richness

starts to decline with increasing latitude beyond the kauri line, and many species with subtropical lineages have southern limits approximating it (Allan 1961; McGlone et al. 2010). This boundary also roughly coincides with the estimated limit of continuous forest cover during the last glacial maxima (Newnham et al. 2013).

Various hypotheses have been proposed to explain the position of the kauri line, variously invoking historical and/or environmental factors. For example, the Pleistocene ratchet hypothesis suggests that current distributions reflect the slow dispersal rates of kauri relative to glacial-interglacial climatic changes (Ogden et al. 1992; McGlone et al. 2017; Lorrey et al. 2018). Alternatively, Clayton-Greene (1978) suggested that the southward movement of kauri is prevented by adverse soils and terrain. Climatic factors, such as low growing season temperatures or more intense frosts south of the kauri line, have also been suggested as potential explanations for its location (Ecroyd 1982; Barton 1985; Ogden & Ahmed 1989).

The impact of climatic stressors on seedling survival often determines a species' range, and changes in these stressors due

to climate change will likely result in range shifts in many plant populations (Giménez-Benavides et al. 2007; Castanha et al. 2013; Landero-Lozada et al. 2019). Frost often impacts seedling survival and species ranges (Sakai & Wardle 1978; Cavanaugh et al. 2014; Pardos et al. 2014). Frosts can reduce soil water availability (Mayr et al. 2006; Zhang et al. 2019) and induce air bubble formation in xylem vessels, which expand on thawing, causing emboli to form (Charrier et al. 2014; Fernández-Pérez et al. 2018). Frosts can also induce cell damage by freezing intracellular solutes in plant tissues or forming ice crystals in cell membranes (Uemura et al. 2006; Wesley-Smith et al. 2015). These events can cause mortality in exposed seedings, especially in open areas, and therefore represent a major filter on the persistence of plant populations in many ecosystems. These frost events, in conjunction with fires, can create feedback loops that perpetuate boundaries in many ecosystems, such as in Brazil (Hoffmann et al. 2019) and South Africa (Duker et al. 2015). Such feedback loops occur especially between open and closed habitats, as frost events are more severe in open areas due to a lower protective effect from canopies (Lusk & Laughlin 2017).

In this study, we evaluated the frost tolerance of tree species associated with the kauri line and compared these tolerances with those of species with more widespread distributions, focussing on patterns between frost tolerance and the ranges of the representative species. Little work has considered the role of frost in potentially setting the southern limits for these species, especially those that can colonise open areas. We used one-off whole-plant freezing tests and electrolyte leakage experiments to evaluate frost tolerance differences between these species and related these to leaf and vascular-based traits (Hofmann et al. 2014; Kreyling et al. 2015).

Methods

The frost tolerances of intact seedlings

We purchased 32 juvenile plants (height excluding pot < 1.3 m) of seven species from two commercial nurseries near the kauri line. *Agathis australis* (Araucariaceae), *Beilschmiedia tarairi* (Lauraceae), *Pseudopanax lessonii* (Araliaceae), and *Vitex lucens* (Lamiaceae) were the kauri line species, and *Podocarpus totara* (Podocarpaceae), *Beilschmiedia tawa* (Lauraceae), and *Lophozonia menziesii* (Nothofagaceae) were the widespread species. These species represented various plant families in each group, while including one taxonomically similar species pair (*B. tarairi/B. tawa*). We purchased the plants on the 19th of May and the 4th of July 2019. We kept them outdoors in Auckland in ambient light and watered to saturation when necessary during acclimation. We moved them into greenhouses at the University of Auckland city campus on the 21st of August 2019 (austral winter), where we conducted our experiments. Plants were watered using the irrigation system in the glasshouses and those that were not at saturation were topped up by hand. The plants were lit by natural light and by 36W/840 (cool white) fluorescent light tubes, which were on from 6 a.m. until 6 p.m. daily.

Using a random stratified process, we divided the seedlings into groups for each treatment (heavy frost, light frost, control), selecting plants so that each treatment group had similar ranges of plant heights. Before the frost treatments, we removed plants with a health rating of fair and below from the experiment (see Appendix S1 in supplementary material). This exclusion removed three *Beilschmiedia tarairi*, three *Vitex lucens*,

seven *Podocarpus totara*, six *Beilschmiedia tawa*, and five *Lophozonia menziesii* from the experiment (Appendix S2). We measured plant heights from the highest living meristem to base with a tape measure, placed each group in a cooling room (13.5 hours at c. 4°C), and started the freezing treatments immediately after removing the plants from the cooling room.

We conducted the freezing treatments by placing the plants in a defrosted chest freezer containing a 13 cm diameter fan to homogenise temperatures. We placed a reference Type T thermocouple, made from TT-T-40-500 insulated wire (Omega Engineering, Stamford, CT, USA) inside the freezer, 15 cm from the freezer base and 5 cm from the freezer wall, with no contact with other surfaces (Lusk et al 2018). Temperatures recorded by the thermocouple were logged at one-second intervals using a C1000x data-logger and this readout was used to maintain consistent temperatures during the experimental periods. We froze the plants in groups of about 14 (randomly selected) due to limitations in freezer space. We conducted the frost tolerance tests from the 10th to the 18th of October and the 5th to the 7th of November 2019.

We placed the plants in the freezer at an initial temperature of 10°C. We then reduced the temperature in the freezer to the target temperatures (−2°C for the light frost treatment and −4°C for the heavy frost treatment) at a rate of approximately 17.5°C per hour; this rapid freezing rate was used for logistical reasons. We held the temperatures within the treatment-specific temperature ranges for five hours and thirty minutes (−2°C to −3°C for the light frost treatment and −4°C to −5°C for the heavy frost treatment). The definitions of light and heavy frosts differ geographically (Hoffmann et al. 2019) and therefore we established these durations and temperatures so that they were consistent with those used for related experiments by Barton (1985) and Fernández-Pérez et al. (2018) and reflected typical frost durations in the field that Barton (1985) recorded within the range of kauri.

After the treatments, we removed the plants from the freezers and returned them to room temperature. For eight weeks following the frost treatments we conducted weekly surveys on the visual health of the plants, scoring each plant's health using a nine-tier categorical scale (Appendix S1). We used an eight week period as it was slightly longer than the 40 days that Barton (1985) used, noting that Barton (1985) observed a range of post-frost damage, mortality, and recovery processes in his samples. Post-freezing effects had stabilised after this time. Additionally, we remeasured the plants' height and the presence and location (e.g. apical, from branches, from the base) of any new growth eight weeks after the treatments for all three treatment groups. We calculated the growth rates of the plants in mm day^{-1} by taking the difference between the heights pre–treatment and those after eight weeks and dividing these by the elapsed time.

We collated each plant's weekly health index scores, height growth rates, and the presence and locations of new growth across all treatment groups. We then used R version 4.3.1 to conduct our analysis (R Core Team 2023). We used non-parametric tests as the sample sizes were small ($n < 12$), and many samples were right-skewed, with few plants showing any height growth. Next, we allocated each seedling to one of four groups based on their height growth or the state of the apical meristem/plant eight weeks after the freezing treatment (1 = plant dead, with no visible living tissue present, 2 = no growth anywhere on the plant, 3 = new growth present on the plant, with no increase in height, 4 = new apical growth and an increase in plant height).

Electrolyte leakage from leaf samples

We conducted the electrolyte leakage tests between the 9th and 13th of March 2020. We extracted 60 leaf segments from each species and allocated five segments to each of 12 test tubes. This method gave us six test tubes as control samples and six that would be frozen per species. We used a cork borer to cut discs of 5 mm diameter from leaves of *B. tarairi*, *B. tawa*, *P. lessonii*, and *V. lucens*. We used different approaches for the smaller leaves of the other species, cutting 5 mm long sections from the narrow laminas of *A. australis* and *P. totara*, and cutting leaves of *L. menziesii* in half.

We then randomly placed the six test tubes to be frozen per species into the middle of the freezer with an internal fan in a corner to homogenise temperatures at 1°C and left them for 40 minutes. We then reduced the freezer temperature over 20 minutes to −4°C and maintained the temperature between −4°C and −5.5°C for 180 minutes. We then raised the temperature to 1°C over one hour by modifying the power supplied to the freezer. We kept the control samples in a fridge at 4°C for the same period. After removal from the fridge/freezer, we transferred the samples to glass tubes containing 25 mL of deionised water. We briefly shook all samples and left them for 15.5 hours before measuring the conductivities of each sample using an Eutech COND 6+ Conductivity meter (Eutech Instruments, Vernon Hills, Ill, USA), stirring the samples before measuring. We then autoclaved the samples to rupture cells and remeasured the conductivities to indicate total electrolyte concentration.

We calculated the relative electrolyte conductivities (proportion of electrolytes leaked) by dividing the conductivities 15.5 hours after freezing by the conductivities following autoclaving (Equation 1).

$$R_c = C_{15.5} / C_A \qquad (1)$$

Where R_c is the relative electrolyte conductivity, $C_{15.5}$ is conductivity after 15.5 hours, and C_A is conductivity following autoclave.

We calculated the index of injury values for each non-control sample using Equation 2 (Flint et al. 1967; Repo et al. 1996) from their associated R_c values obtained from Equation 1, allocating a random control conductivity in the same species/treatment group to each of the treated conductivities.

$$I = 100(R_{ct} − R_{cc}) / (1 − R_{cc}) \qquad (2)$$

Where I is the index of injury value, R_{cc} is the untreated average relative conductivity, and R_{ct} is the relative conductivity of the treatment/frosted sample.

We used a Kruskal Wallis test to assess whether the index of injury values differed among species and a Dunn test to compare these values between species, with the Holm method to correct the *P* values and reduce Type 1 error rates (Holm 1979). We used a linear model to compare the index of injury values to the average health of the plants eight weeks after the freezing treatment and calculated the associated R^2 and *P* values.

Relationships with vessel/tracheid cell widths and specific leaf areas

We collated data from the literature on the vessel (angiosperm) or tracheid (conifer) width, the specific leaf areas (SLAs) of each species, and mean leaf area values of the species sampled

in the trials (Appendix S3). No vessel data were available for *Pseudopanax lessonii*, so we did not consider this species in the following tests. We visually compared these values to the index of injury values and the health scores of the plants after eight weeks. We also visually compared the health scores of the plants after eight weeks to their index of injury values from the electrolyte leakage tests.

Results

The frost tolerance of intact seedlings

Almost all the seedlings (93.65%) assigned to the heavy frost group died within 48 hours of this treatment. Only two *B. tawa* and two *P. totara* individuals survived the treatment and maintained living foliage eight weeks after. In the light frost treatment, the kauri line species suffered more frost damage than the widespread species (Fig. 1). *Agathis australis*, *B. tarairi* and *V. lucens* responded strongly to the light frost treatment, with mortality rates of 40%, 60%, and 22%, respectively at eight weeks after the freezing treatment (Fig. 1). We observed severe and rapid post-treatment leaf loss in *B. tarairi* and *V. lucens*, whereas we primarily observed browning of mature leaves and loss of new growth in treated *A. australis* plants. Recovery occurred in the treated *A. australis* and *V. lucens* seedlings but was largely absent in *B. tarairi*. *Pseudopanax lessonii* generally suffered less severe damage than the other three kauri line species, with high levels of post-treatment recovery in the plants. Some *P. lessonii* seedlings that lost all their living leaves post-frost treatment formed new leaves after four weeks. Post-freezing damage on *P. lessonii* was primarily damage to leaf nodes and meristems and blackening of affected leaves (Appendix S4).

The widespread species consistently showed less frost damage than the kauri line species, with similar health ratings within the group eight weeks after the light frost treatment. The only individual of a widespread species that suffered lethal damage under light frost was a small *L. menziesii* individual that died three weeks following the treatment. We observed moderate damage to *P. totara* individuals immediately following the treatment. However, rapid regrowth was observed from intact meristems, increasing the health ratings of many *P. totara* approximately three weeks after the treatment.

Damage patterns on the plants varied interspecifically. Damage to *L. menziesii* and *P. totara* was concentrated on newly emerged leaves, while leaf damage for *B. tawa* was minimal. However, progressive leaf dieback was observed on single stems of two *B. tawa* plants, with wilting occurring on the plant's apex and progressing downward over approximately 2–3 weeks to various points near the base of the stem. The health status of the control plants of all species did not vary throughout the post-treatment period (Appendix S5).

Patterns of growth following the light frost treatment slightly differed between the two plant groups, with little apical growth occurring in the *A. australis*, *B. tarairi*, and *V. lucens* seedlings in the treatment group compared with the control plants (Fig. 2a). Most of the plants lost their apical meristems in response to the frost treatment. The proportion of individuals showing continued apical growth, however, was higher in *P. lessonii* than in the widespread species (Fig. 2a). Most of the surviving treated seedlings showed some new growth following the frost treatment, with new growth observed at the bases of surviving *B. tarairi*, *V. lucens*, and *P.*
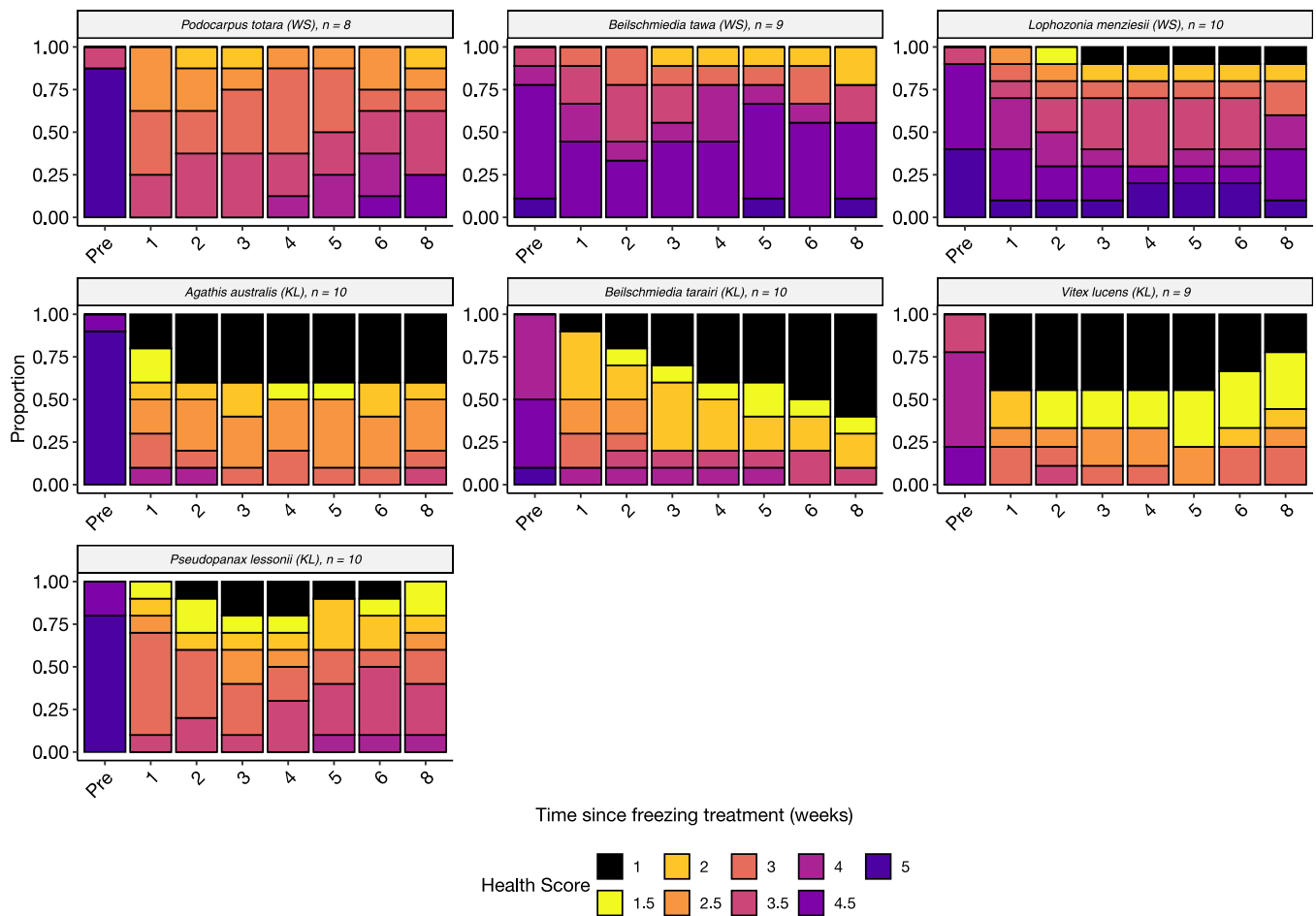
Figure 1. Proportions of health index scores of kauri line (KL) or widespread (WS) plant species in response to an artificial frost treatment with minimum temperatures ranging from −2°C to −3°C over 5.5 hours, where the plants were monitored for eight weeks following the frost treatment. 'Pre' indicates the health distribution of the plants before the frost treatment. We visually scored the plants using a 1–5 health scale, where 5 indicated no visible damage and 1 indicated plants with no visible living foliage. Descriptions of these plant health scores can be found in Appendix S1 with photos in Appendix S4. *n* represents the number of plants in each group.

lessonii individuals. New growth in *A. australis* was observed on branches, frequently originating from nodes between the branches and leaf bases. Most new growth on treated *L. menziesii* and *P. totara* individuals occurred on branches. New growth was present below the dead portions of *B. tawa* stems that underwent wilting due to the frost treatment or on the apexes of surviving stems. The height growth rates of the four kauri line species were significantly lower in the frosted plants than the control plants (Fig. 2b), whereas the growth rates of widespread species did not significantly differ between the frosted and control plants.

Electrolyte leakage from leaf samples and plant trait comparisons

Pseudopanax lessonii, *B. tarairi*, and *V. lucens* had the highest index of injury values (Fig. 3), with *P. lessonii* having the highest value and *B. tawa* and *L. menziesii* having the lowest values. The health scores of the plants after eight weeks were lower in plants that had higher index of injury values in their leaves (Fig. 4a). The health scores were negatively associated with the SLA of most species, regardless of whether they belonged to the kauri line or widespread group (Fig. 4b). Similarly, sindex of injury values tended to be positively associated with the leaf areas of the plants. *Beilschmiedia tarairi* and

V. lucens had much larger leaf areas than the other species and also had high index of injury values from the treated leaf samples (Fig. 4c). The vessel/tracheid diameters appeared to have no relationship to the average health scores of the plants after eight weeks (Fig. 4d). However, widespread species with larger vessels/tracheids had higher health scores in the intact seedling tests than those with narrower vessels/tracheids, while we observed the opposite relationship within the kauri line species (Fig. 4e).

Discussion

Relationship between frost tolerance and southern limits

Our goal in this study was to quantify the frost tolerances of seven New Zealand plant species and relate these to their positions relative to the kauri line, a floristic boundary at 38°S. The relative frost tolerances of the species from both models are highlighted in Table 1.

Both methods showed kauri line species to be less frost tolerant than the widespread species. The loose relationship between the study species' ranges and frost tolerances aligned with the results reported by Bannister (2007) and Sakai and Wardle (1978), where both studies considered the ranges and
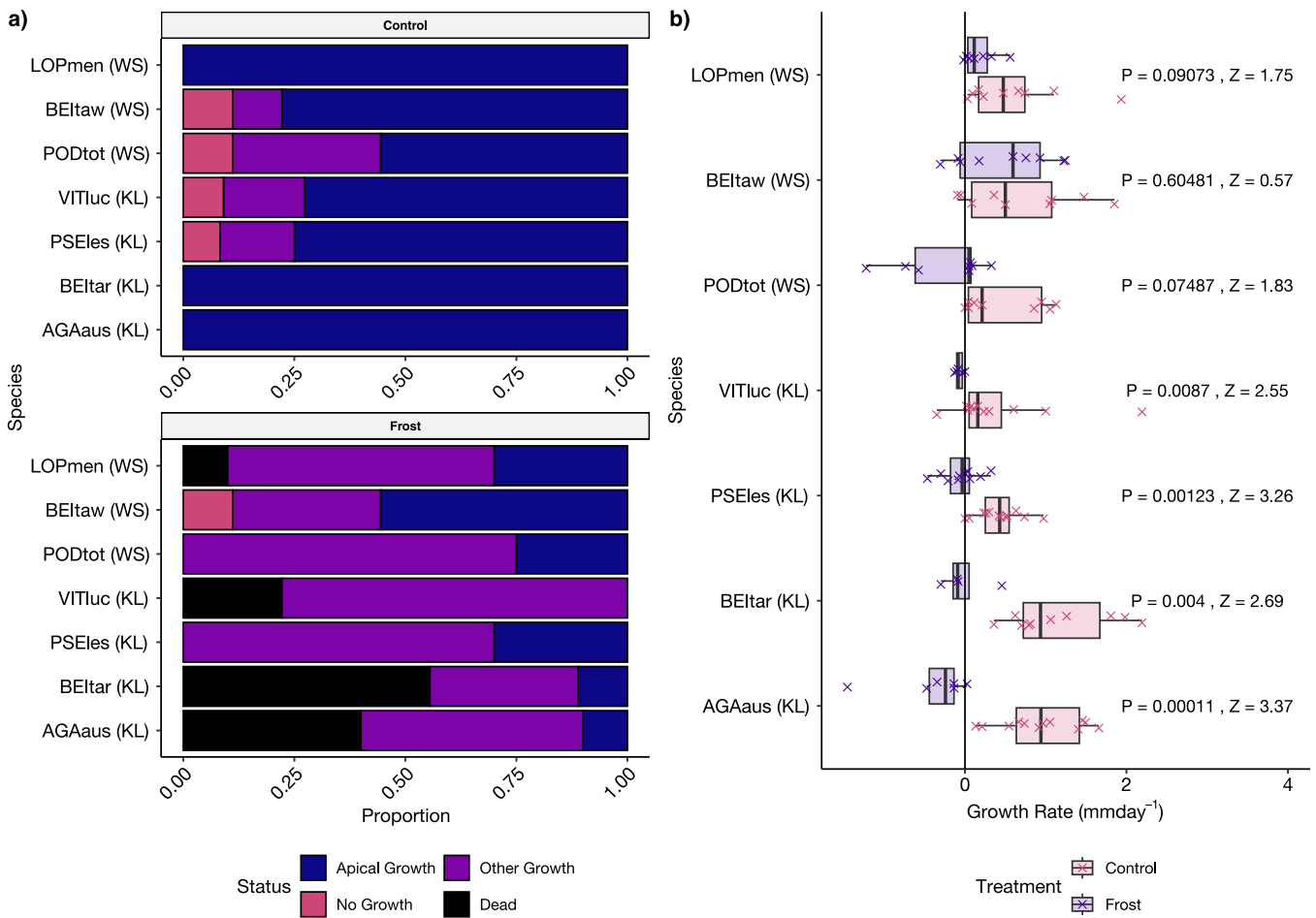
Figure 2. Growth patterns of study species eight weeks after we applied an artificial freezing treatment (frost) or not (control) (A), and the growth rates of the seedlings over eight weeks after the frost treatment (B). Temperatures ranged from −2°C to −3°C, lasting 5.5 hours. 'Dead' indicates individuals that showed no living biomass at the measurement time, and 'no growth' indicates individuals that did not grow over the study period. 'Other growth' indicates plants that showed new growth anywhere on the plant, with no height growth. 'Apical growth' indicates plants that grew in height. We measured the heights from the base to the apical meristem of the plants, excluding the pots. The P and Z values are from a Wilcoxon–Rank sum test, in which we compared the growth rates between the control and treated plants of each species. Species names are as follows: AGAaus = *Agathis australis*, BEItar = *Beilschmiedia tarairi*, PSEles = *Pseudopanax lessonii*, VITluc = *Vitex lucens*, PODtot = *Podocarpus totara*, BEItaw = *Beilschmiedia tawa*, LOPmen = *Lophozonia menziesii*. The kauri line species are labelled as KL, and the widespread species are labelled as WS.
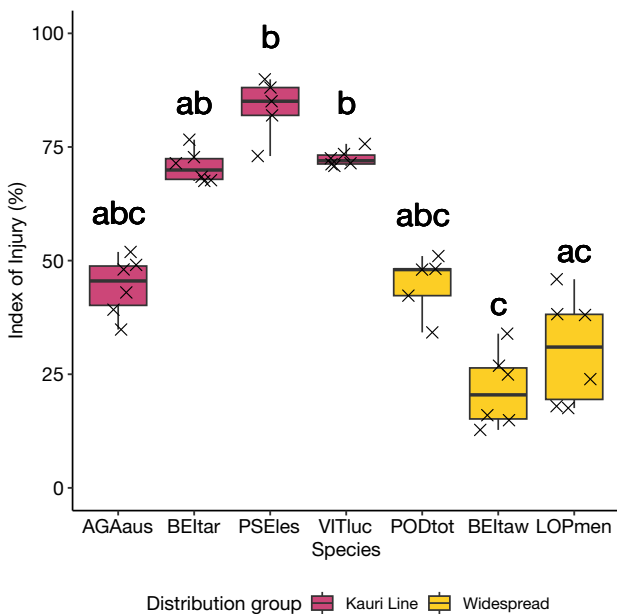
Figure 3. Index of injury values for samples of different plant species following a freezing treatment, where temperatures ranged from between −4°C and −5.5°C for 180 minutes. We took these measurements 15.5 hours after the freezing treatment. We based the index of injury values on the rates of electrolyte leakage of plant samples exposed to the freezing temperature compared to control samples. For species names, see Fig. 2. Kauri line species are those with southern limits approximating 38°S, and widespread species have southern limits further south. P(Kruskall-Wallis) = 4.7 x 10^{-6}. The letters show the groupings formed by a Dunn test, where we used the Holm method to correct the P values.
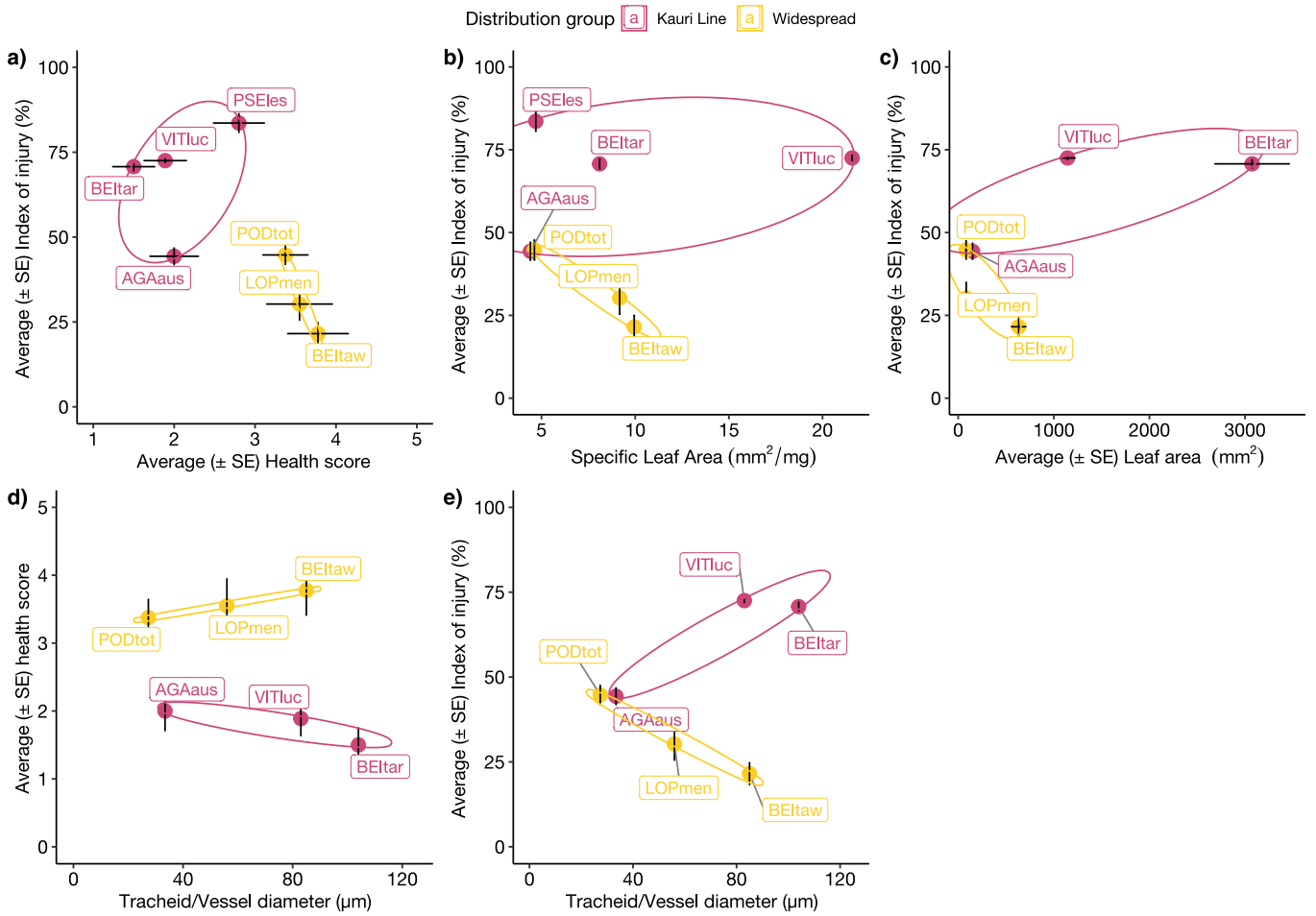
Figure 4. Relationships between various plant traits and their response to freezing treatments. (a) shows the relationship between the average health statuses of whole plants eight weeks after a freezing event (based on a 1–5 scale) and the index of injury values from frozen leaf samples. (b) compares these index of injury values to the specific leaf areas of these species, where we collected data from Mason et al. (2010) and Jager et al. (2015). (c) shows the relationship between the mean leaf areas of the plants and the index of injury values, where we collected data from Richardson et al. (2013), Falster et al. (2015), and Wright et al. (2017), using the TRY plant database (Kattge et al. 2011). No leaf area data were present for *Pseudopanax lessonii* (PSEles), so we did not include it in this graph. (d) shows the relationship between the average vessel diameters of the plants and their health scores after eight weeks. (e) Compares each species' vessel/tracheid diameters and the leaf sample index of injury values. The points are coloured by the species distributions relative to the kauri line at 38°S. Species names are in Fig. 2. The electrolyte leakage method on leaf tissue segments was used to determine the index of injury values. Leaf area is the product of leaf width and length in mm, where the data were taken from the flora of New Zealand database (Allan, 1961). Sources of vessel diameter data are located in the supplementary information (Appendix S3).

Table 1. The relative frost tolerances of the plants, as measured by intact plant tests and through the electrolyte leakage method. The plants are listed in order from lowest to highest frost tolerance.

Relative Frost tolerance	Intact plant tests	Electrolyte leakage tests
Low	*Beilschmiedia tarairi*	*Pseudopanax lessonii*
	Agathis australis	*Beilschmiedia tarairi, Vitex lucens*
	Vitex lucens	
	Pseudopanax lessonii	*Agathis australis*
	Podocarpus totara	*Podocarpus totara*
	Lophozonia menziesii	*Lophozonia menziesii*
High	*Beilschmiedia tawa*	*Beilschmiedia tawa*

frost tolerances of multiple kauri line species. Our observations suggest that frost events may influence seedling establishment and survival at the kauri line species' southern limits. Despite the low mortality of the widespread species in response to the light frost treatment, the heavy frost treatment killed all but four of the widespread individuals, highlighting that the widespread species are still susceptible to intense frosts.

Although we observed a difference between the frost tolerances of the kauri line and widespread species generally, the tolerances of the kauri line species differed and were inconsistent with their southern limits. The southern limits of both *V. lucens* (39.15°S) and *B. tarairi* (38°S) are more southerly than their comparative frost tolerances would predict when compared with *A. australis* (Allan 1961). Furthermore, Sakai and Wardle (1978) reported that *B. tarairi* had a much lower frost tolerance than *A. australis* in a cutting-based study despite sharing a southern limit with *A. australis*. This frost tolerance mismatch may be due to differences in seed dispersal ability; *A. australis* seeds are wind-dispersed and seeds of the other tested kauri line species are bird-dispersed. McGlone et al. (2010) suggest that dispersal ability is the fundamental driver of New Zealand tree species' range sizes and that species with bird-dispersed seeds can occupy wider ranges than those with wind-dispersed seeds. Dispersal may have contributed to *P. totara*'s extensive range relative to *A. australis*, despite it being the least frost tolerant of the widespread species in our tests. This mismatch may have been promoted by legacy effects of glacial-interglacial cycles, whereby kauri and other wind-dispersed species may not have achieved their former ranges due to their dispersal rates being too slow to keep pace with interglacial-glacial cycles (the Pleistocene rachet) (McGlone et al. 2016). Alternatively, climatic conditions observed during postglacial climatic warming, such as cloudy summers, may have delayed the southward movement of kauri (Ogden et al. 1992). The widespread species were generally more frost tolerant than the kauri line species, but the tolerance-southern limit relationship within this group was less clear than in the kauri line species. For example, the southern limit of the frost tolerant *B. tawa* is c. 42°S, but is diffuse, with potential refugia further south (e.g. Kaikoura at 42.4°S) (Vanderhoorn 2020), and is nonetheless further north than the limits of the other widespread species, which occur throughout New Zealand (Allan 1961). This difference could have arisen from different frost hardening potential between the three species (Sun & Sweet 1996), regional differences in frost tolerance (Hawkins et al. 1991), legacy effects of the last glacial maxima (Ogden et al. 1992; Steward et al. 2003), the high vessel diameters and leaf areas of *B. tawa*, or interspecific differences in competitive ability.

Electrolyte leakage versus whole plant tests

Unlike the widespread species, kauri line species' resistance to frost-induced electrolyte leakage in leaf tissue did not necessarily translate into whole-plant frost tolerance. For example, *P. lessonii*, which was relatively frost tolerant in the whole plant tests, had the highest index of injury value after 15.5 hours. Likewise, *A. australis* had a lower-than-expected value, which did not differ markedly from that of *P. totara*. This pattern is consistent with the results of Sakai and Wardle (1978), who found that both species had frost tolerances at −7°C. Conversely, Hawkins et al. (1991) reported far lower rates of frost damage on *P. totara* seedlings than Barton (1985) found in *A. australis* seedlings at the same temperature (−5°C), which is consistent with our results from the whole plant tests. Factors such as

xylem embolism formation susceptibility could contribute to this disconnect in comparative frost tolerance between the two species. *Agathis australis* stems have wider tracheids than those of *P. totara* and other New Zealand conifer species that grow further south than *A. australis*, such as *Phyllocladus trichomioides* (Patel 1967). Pittermann et al. (2006), through a study focussing on the hydraulic conductivities of various gymnosperms, reported that low pressures were needed to induce a 50% loss in *A. australis* stem and root conductivity relative to the other tested species. These results suggest that freezing events may induce embolism formation in *A. australis* seedlings, causing further damage and inhibiting their recovery potential (Patel 1967, 1968; Tyree et al. 1998; Mayr et al. 2003; Laughlin et al. 2020).

The higher frost tolerance of *B. tawa* relative to *V. lucens*, despite their similar vessel diameters (Patel 1974, 1988), may reflect differences in leaf frost tolerance rather than vessel embolism sensitivity. The higher index of injury values we reported for *V. lucens* compared with *B. tawa* support this difference, where *B. tawa* leaf traits (e.g. small, narrow leaves) may protect their leaves from freezing damage (Lusk et al. 2018). These changes in perceived frost tolerances with the chosen testing method suggest that frost tolerance extends beyond leaf damage in plants, and that both leaf and vessel frost tolerances must be considered when assessing seedling frost tolerances. Therefore, studies focusing on whole plants are preferable to those using cuttings, which should only be used as a screening tool. Cutting-based methods do not consider the ability of different species to recover from frost events, their sensitivity to xylem embolisms, or the role of other structures such as stems that may provide support or protection from frosts (Fernández-Pérez et al. 2018; Ambroise et al. 2020). While our results demonstrate a relationship between frost tolerance and the southern limits of the plant species, the species, based on their traits, are being affected by different mechanisms. These traits (e.g. wide vessels) present 'weaknesses' against frosts that the artificial intact seedling freezing tests in this study exploited, resulting in the observed differences.

Lab-based versus field-based interpretations of frost tolerance

Although we found an association between the southern limits of the tested species and their frost tolerances, we cannot determine the role that frosts play in setting the southern limits of these species without *in situ* tests. Local microenvironments are often highly modified by canopy effects (Wagner et al. 2011), where canopies can increase ground-level temperatures and prevent frosts (Hoffmann et al. 2019), especially in New Zealand, where most trees are evergreen (Lusk & Laughlin 2017). Therefore, sheltered sites immediately south of the kauri line are unlikely to experience severe frosts that match the temperatures used in this study. However, species with frost-intolerant and shade-intolerant seedlings, such as *A. australis*, are unlikely to benefit from these canopy effects as they typically occupy more open areas (Bieleski 1959; Ogden 1985).

We observed rapid recovery in many *P. lessonii, V. lucens* and *P. totara,* suggesting that these are relatively tolerant of our light frost treatments. Species differ in their ability to recover from frosts due to differences in carbohydrate storage capacity and other physiological adaptations (Landhäusser & Lieffers 1997; Hekneby et al. 2006; Ladwig et al. 2019). These opportunities to recover may not be present in the field, as individuals experience stresses such as shading and

herbivory that were absent in our study. Furthermore, these species are now likely at a major competitive disadvantage compared to frost-tolerant species, especially if they suffer hidden forms of sub-lethal damage such as reduced hydraulic conductivity (Stoddard et al. 2006). Field-based studies of post-frost recovery are vital to improve our interpretation of interspecific variation in frost tolerances.

In temperate regions, traits that increase plant frost tolerance, such as smaller leaves or narrow xylem conduits, may reduce their productivity and competitive ability in frost-free habitats and limit the northward expansion of frost-tolerant species (Schreiber et al. 2013; Lusk et al. 2019; Gast et al. 2020). For example, species that have large leaf surface areas (e.g. *B. tarairi*) can efficiently capture light and out-compete neighbours with smaller leaves, especially in heavily shaded areas (Clark & Sturman 2009; Lusk et al. 2012). However, these large leaves cool faster than smaller leaves, making them more susceptible to frost damage. This confines many large-leaved evergreen species in New Zealand to areas where frosts are rare (Lusk et al. 2018). However, many coniferous and small-leaved species, such as *Coprosma arborea*, *P. totara*, and *Dacrycarpus dacrydioides*, are relatively common components of northern forests (Allan 1961). More field-based analysis on the population dynamics of these species and their competitive potentials in various northern microclimates would be beneficial in examining how these species manage to occupy these habitats successfully and minimise the effects of competition.

Across much of New Zealand, frosts are likely to reduce in intensity and frequency under climate change (Clark & Sturman 2009), which may alter the distributions of the species we studied. For example, *P. lessonii*, with an intermediate frost tolerance, is already invasive outside its current range. Hopkins (2010) observed individuals growing at 41.2°S. Its invasive potential may increase with climate change induced increases in mild frosts that do not impair *P. lessonii* seedling survival or competitive ability. Furthermore, *P. lessonii* has a shorter pre-reproductive period than the other three species, which are long–lived trees, and will likely be able to shift its range in response to rapid human-mediated climate change more successfully than the other three species. These shifts may allow kauri line species, especially those like *P. lessonii*, to outcompete more frost tolerant species. These species may, therefore, lose vital refugia in which they have an advantage. Our study suggests that frost has a role in setting the position of the kauri line. More field-based studies that are focused directly on the intensity of frosts in forested areas near the kauri line and their effects on exposed seedlings will be a logical next step to examine their influence on the position of the kauri line.

Additional information and declarations

Author contributions: TE, BB, and CM conceptualised the project and devised the methods. TE undertook the experimental work and the data analysis. TE wrote the initial draft, and all authors contributed to subsequent reviews and editing.

Data and code availability: data and code for the study are available via Figshare: https://doi.org/10.6084/m9.figshare.25885369.v2

Ethics: no ethics approvals were required to undertake this work.

Conflicts of interest: the authors declare no conflicts of interest.

References

Allan HH 1961. Flora of New Zealand. Volume 1. Wellington, Government Printer. 1085 p.

Ambroise V, Legay S, Guerriero G, Hausman J-F, Cuypers A, Sergeant K 2020. The roots of plant frost hardiness and tolerance. Plant and Cell Physiology 61(1): 3–20.

Antonelli A 2017. Biogeography: drivers of bioregionalization. Nature Ecology & Evolution 1(4): 0114.

Bannister P 2007. Godley review: a touch of frost? Cold hardiness of plants in the southern hemisphere. New Zealand Journal of Botany 45(1): 1–33.

Bartlett JL 1976. Rare and unusual plants north of the kauri line. New Zealand Journal of Botany 14(2): 135–150.

Barton IL 1985. Winter frost and its effect on kauri (*Agathis australis*) seedlings. New Zealand Journal of Forestry 30(1): 94–101.

Bieleski RL 1959. Factors affecting growth and distribution of kauri (*Agathis australis* Salisb.) II. effect of light intensity on seedling growth. Australian Journal of Botany 7(3): 268–278.

Castanha C, Torn MS, Germino MJ, Weibel B, Kueppers LM 2013. Conifer seedling recruitment across a gradient from forest to alpine tundra: effects of species, provenance, and site. Plant Ecology & Diversity 6(3–4): 307–318.

Cavanaugh KC, Kellner JR, Forde AJ, Gruner DS, Parker JD, Rodriguez W, Feller IC 2014. Poleward expansion of mangroves is a threshold response to decreased frequency of extreme cold events. Proceedings of the National Academy of Sciences 111(2): 723–727.

Charrier G, Charra-Vaskou K, Kasuga J, Cochard H, Mayr S, Améglio T 2014. Freeze-thaw stress: effects of temperature on hydraulic conductivity and ultrasonic activity in ten woody angiosperms. Plant Physiology 164(2): 992–998.

Clark A, Sturman J 2009. Recent frost trends for New Zealand. Wellington, New Zealand, Ministry of Agriculture and Forestry. 72 p.

Clayton-Greene KA 1978. Aspects of the distribution of certain indigenous woody species in the Waikato District, New Zealand. Journal of the Royal Society of New Zealand 8(3): 283–291.

Cockayne L 1917. Notes of New Zealand floristic botany, including descriptions of new species. Transactions and Proceedings of the New Zealand Institute 49: 56–65.

Duker R, Cowling RM, du Preez DR, van der Vyver ML, Weatherall-Thomas CR, Potts AJ 2015. Community-level assessment of freezing tolerance: frost dictates the biome boundary between Albany subtropical thicket and Nama-Karoo in South Africa. Journal of Biogeography 42(1): 167–178.

Ecroyd CE 1982. Biological flora of New Zealand 8. *Agathis australis* (D. Don) Lindl. (Araucariaceae) kauri. New Zealand Journal of Botany 20(1): 17–36.

Esler KJ, von Staden L, Midgley GF 2015. Determinants of the fynbos/succulent karoo biome boundary: insights from a reciprocal transplant experiment. South African Journal of Botany 101: 120–128.

Ettinger AK, HilleRisLambers J 2013. Climate isn't everything: competitive interactions and variation by life stage will also affect range shifts in a warming world. American Journal of Botany 100(7): 1344–1355.

Falster DS, Duursma RA, Ishihara MI, Barneche DR, FitzJohn RG, Vårhammar A, Aiba M, Ando M, Anten N, Aspinwall MJ, Baltzer JL, Baraloto C, Battaglia M, Battles JJ, Bond-Lamberty B, van Breugel M, Camac J, Claveau Y, Coll L, Dannoura M, York RA, et al. 2015. BAAD: a biomass and allometry database for woody plants. Ecology 96(5): 1445–1445.

Fernández-Pérez L, Villar-Salvador P, Martínez-Vilalta J, Toca A, Zavala MA 2018. Distribution of pines in the Iberian Peninsula agrees with species differences in foliage frost tolerance, not with vulnerability to freezing-induced xylem embolism. Tree Physiology 38(4): 507–516.

Flint HL, Boyce BR, Beattie DJ 1967. Index of injury - a useful expression of freezing injury to plant tissues as determined by the electrolytic method. Canadian Journal of Plant Science 47(2): 229–230.

Gast A, Römermann C, Bucher SF 2020. Seasonal variation and trade-off between frost resistance and photosynthetic performance in woody species. Photosynthetica 58: 331–340.

Giménez-Benavides L, Escudero A, Iriondo JM 2007. Local adaptation enhances seedling recruitment along an altitudinal gradient in a high mountain mediterranean plant. Annals of Botany 99(4): 723–734.

Hawkins BJ, Sweet GB, Greer DH, Bergin DO 1991. Genetic variation in the frost hardiness of *Podocarpus totara*. New Zealand Journal of Botany 29(4): 455–458.

Hekneby M, Antolín MC, Sánchez-Díaz M 2006. Frost resistance and biochemical changes during cold acclimation in different annual legumes. Environmental and Experimental Botany 55(3): 305–314.

Hoffmann WA, Flake SW, Abreu RCR, Pilon NAL, Rossatto DR, Durigan G 2019. Rare frost events reinforce tropical savanna–forest boundaries. Journal of Ecology 107(1): 468–477.

Hofmann M, Jager M, Bruelheide H 2014. Relationship between frost hardiness of adults and seedlings of different tree species. IForest - Biogeosciences and Forestry 7(5): 282–288.

Holm S 1979. A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics 6(2): 65–70.

Hopkins C 2010. Out-of-place native plants and environmental restoration. Wellington Botanical Society Bulletin 52(1): 47–49.

Jager MM, Richardson SJ, Bellingham PJ, Clearwater MJ, Laughlin DC 2015. Soil fertility induces coordinated responses of multiple independent functional traits. Journal of Ecology 103(2): 374–385.

Kattge J, Díaz S, Lavorel S, Prentice IC, Leadley P, Bönisch G, Garnier E, Westoby M, Reich PB, Wright IJ, Cornelissen JHC, Violle C, Harrison SP, Van Bodegom PM, Reichstein M, Enquist BJ, Soudzilovskaia NA, Ackerly DD, Anand M, Atkin O, Wirth C, et al. 2011. TRY – a global database of plant traits. Global Change Biology 17(9): 2905–2935.

Kreyling J, Schmid S, Aas G 2015. Cold tolerance of tree species is related to the climate of their native ranges. Journal of Biogeography 42(1): 156–166.

Ladwig LM, Collins SL, Krofcheck DJ, Pockman WT 2019. Minimal mortality and rapid recovery of the dominant shrub *Larrea tridentata* following an extreme cold event in the northern Chihuahuan Desert. Journal of Vegetation Science 30(5): 963–972.

Landero-Lozada S, Toledo-Aceves T, López-Barrera F, Sosa VJ, Ramírez-Marcial N 2019. Early establishment of endangered and valuable tree species in cloud forest restoration plantings. Revista Mexicana de Biodiversidad 90: e902550.

Landhäusser SM, Lieffers VJ 1997. Seasonal changes in carbohydrate storage and regrowth in rhizomes and stems of four boreal forest shrubs: applications in *Picea glauca* understorey regeneration. Scandinavian Journal of Forest Research 12(1): 27–32.

Laughlin DC, Delzon S, Clearwater MJ, Bellingham PJ, McGlone MS, Richardson SJ 2020. Climatic limits of temperate rainforest tree species are explained by xylem embolism resistance among angiosperms but not among conifers. The New Phytologist 226(3): 727–740.

Liu Y, Xu X, Dimitrov D, Pellissier L, Borregaard MK, Shrestha N, Su X, Luo A, Zimmermann NE, Rahbek C, Wang Z 2023. An updated floristic map of the world. Nature Communications 14(1): 2990.

Lorrey AM, Boswijk G, Hogg A, Palmer JG, Turney CSM, Fowler AM, Ogden J, Woolley J-M 2018. The scientific value and potential of New Zealand swamp kauri. Quaternary Science Reviews 183: 124–139.

Lusk CH, Laughlin DC 2017. Regeneration patterns, environmental filtering and tree species coexistence in a temperate forest. New Phytologist 213(2): 657–668.

Lusk CH, Pérez-Millaqueo MM, Saldaña A, Burns BR, Laughlin DC, Falster DS 2012. Seedlings of temperate rainforest conifer and angiosperm trees differ in leaf area display. Annals of Botany 110(1): 177–188.

Lusk CH, Clearwater MJ, Laughlin DC, Harrison SP, Prentice IC, Nordenstahl M, Smith B 2018. Frost and leaf-size gradients in forests: global patterns and experimental evidence. New Phytologist 219(2): 565–573.

Lusk CH, Grierson ERP, Laughlin DC 2019. Large leaves in warm, moist environments confer an advantage in seedling light interception efficiency. New Phytologist 223(3): 1319–1327.

Mason NWH, Peltzer DA, Richardson SJ, Bellingham PJ, Allen RB 2010. Stand development moderates effects of ungulate exclusion on foliar traits in the forests of New Zealand. Journal of Ecology 98(6): 1422–1433.

Mayr S, Gruber A, Bauer H 2003. Repeated freeze-thaw cycles induce embolism in drought stressed conifers (Norway spruce, stone pine). Planta 217(3): 436–441.

Mayr S, Hacke U, Schmid P, Schwienbacher F, Gruber A 2006. Frost drought in conifers at the alpine timberline: xylem dysfunction and adaptations. Ecology 87(12): 3175–3185.

McGlone MS 1985. Plant biogeography and the late Cenozoic history of New Zealand. New Zealand Journal of Botany 23(4): 723–749.

McGlone MS, Richardson SJ, Jordan GJ 2010. Comparative biogeography of New Zealand trees: species richness, height, leaf traits and range sizes. New Zealand Journal of Ecology 34(1): 137–151.

McGlone M, Buitenwerf R, Richardson S 2016. The formation of the oceanic temperate forests of New Zealand. New Zealand Journal of Botany 54(2): 128–155.

McGlone MS, Richardson SJ, Burge OR, Perry GLW, Wilmshurst JM 2017. Palynology and the ecology of the New Zealand conifers. Frontiers in Earth Science 5, 94.

Newnham R, McGlone M, Moar N, Wilmshurst J, Vandergoes

M 2013. The vegetation cover of New Zealand at the last glacial maximum. Quaternary Science Reviews 74: 202–214.

Ogden J 1985. An introduction to plant demography with special reference to New Zealand trees. New Zealand Journal of Botany 23(4): 751–772.

Ogden J, Ahmed M 1989. Climate response function analyses of kauri (Agathis australis) tree-ring chronologies in northern New Zealand. Journal of the Royal Society of New Zealand 19(2): 205–221.

Ogden J, Perry GLW 2023. Ranges of woody plant species and ferns on forested elevational gradients on Aotea-Great Barrier Island, New Zealand: the role of zones of permanent and temporary establishment. New Zealand Journal of Ecology 47(1): 3512.

Ogden J, Wilson A, Hendy C, Newnham RM, Hogg AG 1992. The late Quaternary history of kauri (Agathis australis) in New Zealand and its climatic significance. Journal of Biogeography 19(6): 611.

Pardos M, Climent J, Almeida H, Calama R 2014. The role of developmental stage in frost tolerance of Pinus pinea L. seedlings and saplings. Annals of Forest Science 71(5): 551–562.

Patel RN 1967. Wood anatomy of Podocarpaceae indigenous to New Zealand: 2. Podocarpus. New Zealand Journal of Botany 5(3): 307–321.

Patel RN 1968. Wood anatomy of Cupressaceae and Araucariaceae indigenous to New Zealand. New Zealand Journal of Botany 6(1): 9–18.

Patel RN 1974. Wood anatomy of the dicotyledons indigenous to New Zealand 5. Verbenaceae. New Zealand Journal of Botany 12(1): 33–44.

Patel RN 1988. Wood anatomy of the dicotyledons indigenous to New Zealand 17. Tiliaceae. New Zealand Journal of Botany 26(3): 337–343.

Pittermann J, Sperry JS, Wheeler JK, Hacke UG, Sikkema EH 2006. Mechanical reinforcement of tracheids compromises the hydraulic efficiency of conifer xylem. Plant, Cell & Environment 29(8): 1618–1628.

R Core Team 2023. R: a language and environment for statistical computing. Version 4.3.1. Vienna, Austria, R foundation for statistical computing. http://www.R-project.org/

Repo T, Hänninen H, Kellomäki S 1996. The effects of long-term elevation of air temperature and CO on the frost hardiness of Scots pine. Plant, Cell & Environment 19(2): 209–216.

Richardson SJ, Allen RB, Buxton RP, Easdale TA, Hurst JM, Morse CW, Smissen RD, Peltzer DA 2013. Intraspecific relationships among wood density, leaf structural traits and environment in four co-occurring species of Nothofagus in New Zealand. PLoS One 8(3): e58878.

Sakai A, Wardle P 1978. Freezing resistance of New Zealand trees and shrubs. New Zealand Journal of Ecology 1: 51–61.

Schreiber SG, Ding C, Hamann A, Hacke UG, Thomas BR, Brouard JS 2013. Frost hardiness vs. growth performance in trembling aspen: an experimental test of assisted migration. Journal of Applied Ecology 50(4): 939–949.

Sexton JP, McIntyre PJ, Angert AL, Rice KJ 2009. Evolution and ecology of species range limits. Annual Review of Ecology, Evolution, and Systematics 40(1): 415–436.

Slik JWF, Aiba S-I, Bastian M, Brearley FQ, Cannon CH, Eichhorn KAO, Fredriksson G, Kartawinata K, Laumonier Y, Mansor A, Marjokorpi A, Meijaard E, Morley RJ, Nagamasu H, Nilus R, Nurtjahya E, Payne J, Permana A, Poulsen AD, Raes N, Riswan S, van Schaik CP, Sheil D, Sidiyasa K, Suzuki E, van Valkenburg JLCH, Webb CO, Wich S, Yoneda T, Zakaria R, Zweifel N 2011. Soils on exposed sunda shelf shaped biogeographic patterns in the equatorial forests of Southeast Asia. Proceedings of the National Academy of Sciences 108(30): 12343–12347.

Steward GA, Bergin DO, Winstanley WJ 2003. Two records of kauri regeneration from trees planted south of the species' natural range. New Zealand Journal of Forestry Science 33(1): 3–9.

Stoddard FL, Balko C, Erskine W, Khan HR, Link W, Sarker A 2006. Screening techniques and sources of resistance to abiotic stresses in cool-season food legumes. Euphytica 147(1): 167–186.

Sun OJ, Sweet GB 1996. Comparison of frost tolerance of Nothofagus solandri var. cliffortioides (Hook.f.) Poole and Nothofagus menziesii (Hook.f.) Oerst. New Zealand Journal of Botany 34(2): 273–278.

Tyree MT, Patiño S, Becker P 1998. Vulnerability to drought-induced embolism of Bornean heath and dipterocarp forest trees. Tree Physiology 18(8–9): 583–588.

Uemura M, Tominaga Y, Nakagawara C, Shigematsu S, Minami A, Kawamura Y 2006. Responses of the plasma membrane to low temperatures. Physiologia Plantarum 126(1): 81–89.

Vanderhoorn J 2020. The ghosts of forests past: mapping the ghost taxon Beilschmiedia tawa through species distribution modelling and co-occurring species. Unpublished MSc. thesis, University of Auckland, Auckland, New Zealand.

Wagner S, Fischer H, Huth F 2011. Canopy effects on vegetation caused by harvesting and regeneration treatments. European Journal of Forest Research 130(1): 17–40.

Wardle P 1963. Evolution and distribution of the New Zealand flora, as affected by Quaternary climates. New Zealand Journal of Botany 1(1): 3–17.

Wesley-Smith J, Walters C, Pammenter NW, Berjak P 2015. Why is intracellular ice lethal? A microscopical study showing evidence of programmed cell death in cryo-exposed embryonic axes of recalcitrant seeds of Acer saccharinum. Annals of Botany 115(6): 991–1000.

Wright IJ, Dong N, Maire V, Prentice IC, Westoby M, Díaz S, Gallagher RV, Jacobs BF, Kooyman R, Law EA, Leishman MR, Niinemets Ü, Reich PB, Sack L, Villar R, Wang H, Wilf P 2017. Global climatic drivers of leaf size. Science 357(6354): 917–921.

Zhang W, Jiang Z, Zhao H, Feng F, Cai J 2019. Frost fatigue response to simulated frost drought using a centrifuge in Acer mono Maxim. Physiologia Plantarum 166(2): 677–687.